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ORIGINAL RESEARCH

Comparing the Relationships of Genetically Proxied PCSK9 Inhibition With Mood Disorders, Cognition, and Dementia Between Men and Women: A Drug-Target Mendelian Randomization Study

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BACKGROUND: PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors are important therapeutic options for reducing cardiovascular disease risk; however, questions remain regarding potential differences in the neuropsychiatric impact of long-term PCSK9 inhibition between men and women.

METHODS AND RESULTS: Using PCSK9 gene single-nucleotide polymorphisms from European ancestry–based genome-wide association studies of low-density lipoprotein cholesterol (N=1 320 016), circulating PCSK9 protein levels (N=10 186), tissue-specific PCSK9 gene expression, sex-specific genome-wide association studies of anxiety, depression, cognition, insomnia, and dementia (ranging from 54 321 to 194 174), we used drug-target inverse variance–weighted Mendelian randomization (MR) and complementary MR methods (MR Egger, weighted median, and weighted mode) to investigate potential neuropsychiatric consequences of genetically proxied PCSK9 inhibition in men and women. We failed to find evidence surpassing correction for multiple comparisons of relationships between genetically proxied PCSK9 inhibition and the risk for the 12 neuropsychiatric end points in either men or women. Drug-target analyses were generally well-powered to detect effect estimates at several hypothesized thresholds for both combined-sex and sex-specific end points, especially analyses using PCSK9 instruments derived from protein and expression quantitative trait loci. Further, MR estimates across complementary MR methods and additional models using genetic instruments derived from circulating PCSK9 protein levels and tissue-specific *PCSK9* expression were in alignment, strengthening causal inference.

CONCLUSIONS: Genetically proxied PCSK9 inhibition showed a neutral neuropsychiatric side effect profile with no major sex-specific differences. Given statistical power considerations, replication with larger samples, as well as data from other ancestral populations, are necessary. These findings may have important clinical implications for lipid-lowering drug-prescribing practices and side effect monitoring of approved and future PCSK9 therapies.

Key Words: Alzheimer disease ■ cholesterol ■ cognition ■ dementia ■ depression ■ low-density lipoprotein ■ Mendelian randomization ■ PCSK9

Hyperlipidemia is a disease marked by a high concentration of lipids in the blood and has been known for some time to play a role in the pathogenesis of diseases of the cardiovascular and neurological system, including myocardial infarction, stroke, and sudden cardiac death.[1–6] While hyperlipidemia includes cases

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CLINICAL PERSPECTIVE

What Is New?

- PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors exhibit a safe neuropsychiatric outcome profile, which is not significantly different in male and female samples.

What Are the Clinical Implications?

- Genetic evidence suggests that PCSK9 inhibitors do not pose a significant risk for adverse cognitive outcomes reported in some studies of statins, and concern regarding cognitive side effects contributing to underuse of PCSK9 inhibitors may be unfounded.

Nonstandard Abbreviations and Acronyms

CRISPR	clustered regularly interspaced short palindromic repeats
eQTL	expression quantitative trait loci
GLGC	Global Lipids Genetics Consortium
GTEx	Genotype-Tissue Expression
InSIDE	Instrument Strength Independent of Direct Effect
IV	instrumental variable
IVW	inverse variance–weighted
MR	Mendelian randomization
PCSK9	proprotein convertase subtilisin/kexin type 9
PCSK9i	proprotein convertase subtilisin/kexin type 9 inhibitor
pQTL	protein quantitative trait loci
TPM	transcripts per million

of elevated blood triglycerides, hypercholesterolemia is marked by elevated blood low-density lipoprotein cholesterol (LDL-C). Hyperlipidemia and hypercholesterolemia are among the leading causes of cardiovascular disease, the leading cause of death worldwide, through their role in the formation of atherosclerotic plaques.[7–11]

Pharmacologic modification of atherogenic lipoprotein levels by lipid-lowering therapies such as statins[12] and *PCSK9* (proprotein convertase subtilisin/kexin type 9) (monoclonal antibody inhibitors (alirocumab and evolocumab)[13] are considered effective approaches to reducing cardiovascular disease risk.[14] New pharmaceutical approaches to PCSK9 inhibition (PCSK9i) are also being developed. Inclisiran, a small inhibitory RNA molecule which inhibits PCSK9 via the RNA interference (RNAi) pathway[15] has recently been approved by the European Commission and US Food and Drug Administration to be used in combination with statins and diet to lower LDL-C levels in adults with hypercholesterolemia or atherosclerosis,[15–18] and recent preclinical work using CRISPR gene editing to induce a PCSK9 knockout genotype reported long-term cholesterol reduction in nonhuman primates.[19,20]

While short-term clinical trials investigating the neuropsychiatric impact of PCSK9 monoclonal antibodies reported no major adverse neuropsychiatric events among study participants,[13,21–23] there remains concern about the potential adverse neuropsychiatric impact of PCSK9i therapy, owing in part to potential cognitive effects that have been observed in some studies of statins,[24,25] and in vitro and in vivo studies implicating *PCSK9* in a range of neural processes.[26,27] For example, PCSK9 has been shown to be involved in neuronal differentiation, apoptosis, astrocytes, and glial cell activation; neuronal *PCSK9* expression has been shown to be upregulated in adult brains during disease states, including Alzheimer disease (AD), alcohol use disorder, ischemic stroke, and mood disorders[26–28]; and Mendelian randomization (MR) analyses have suggested that genetically proxied long-term PCSK9i is associated with increased risk for depression in individuals of European ancestry.[29]

As new PCSK9i therapeutics become approved for clinical use, it is important to examine and understand their long-term efficacy and side effect profiles in populations representative of the patients who will use the therapeutics, including any potential differences between those of men and women.[29,30] Neuropsychiatric disorder prevalence and risk profiles are known to differ between men and women.[31,32] Women are twice as likely as men to be diagnosed with depression,[31] are more likely to present with most major subtypes of depression, and report greater symptom severity.[33–36] Given sex-related differences in mood disorders and dementia risk, it is important to investigate any potential sex-specific effects of PCSK9i, and while previous short-term clinical trials and genetics-based studies have failed to find evidence of large-scale neuropsychiatric effects related to PCSK9i,[37–40] any potential differences in risk between men and women have not been investigated. Although randomized controlled trials (RCTs) remain the gold standard for assessing causal relationships between risk factors and disease outcomes,[41,42] an RCT investigating the long-term and potential sex-specific impact of PCSK9i on neuropsychiatric disorders would be challenging because of the recency of PCSK9i approval, and consequently long-term neuropsychiatric data from RCTs are not yet available. In addition, despite improved sex equity in RCTs over the past 20 years, there remains sex bias within clinical trials that may not capture important differences between men and women.[30]

Therefore, the present study sought to determine whether there is genetic evidence of a sex-specific impact of PCSK9i therapy on mood disorders, cognition, or dementia. We employed drug-target MR, a recent extension of MR using single-nucleotide polymorphisms (SNPs) associated with druggable gene targets (ie, SNPs within or near the *PCSK9* gene locus),[43] to genetically proxy and evaluate the lifelong impact on outcomes of interest caused by pharmacological modulation of the gene target (ie, inhibition of *PCSK9*),[43] and summary-level genome-wide association study (GWAS) data in men, women, and in combined-sex samples. In addition to instrumenting genetically proxied PCSK9i in LDL-C levels (the primary physiological response to pharmacological PCSK9i), we also leveraged recently released GWAS data on circulating PCSK9 protein levels and cerebral cortex– and liver-specific *PCSK9* gene expression data in order to better genetically model the mechanisms of action for the anti-PCSK9 monoclonal antibodies[44] and inclisiran,[15] respectively.

METHODS

Code Availability

Code is available from the authors upon reasonable request. The current study used the TwoSampleMR R package (https://mrcieu.github.io/TwoSampleMR/). Figures 1 and 2 were created using BioRender.com. The mRnd Shiny App used for power calculations is available at https://shiny.cnsgenomics.com/mRnd/.

iData Availability

All exposure instruments required to replicate the analyses are located in the Supplemental Tables. The current study used publicly available GWAS summary statistics. The neuropsychiatric endpoint data for men, women, and combined-sex samples are available from the Neale Lab repository (http://www.nealelab.is/uk-biobank). The Global Lipids Genetics Consortium (GLGC) LDL-C data are available from the GLGC downloads page (http://csg.sph.umich.edu/willer/public/glgc-lipids2021/). GWAS results of circulating PCSK9 protein levels are available at https://zenodo.org/record/5643551. Genotype-Tissue Expression (GTEx) expression quantitative trait loci (eQTL) data are available from its respective downloads page (https://gtexportal.org/home/datasets). MetaBrain eQTL data are available at: https://metabrain.nl/.

Approval and Data Sources

Figure 1 provides a study overview. The present study used publicly available, summary-level genome-wide association study (GWAS) data. The data sources used (UK Biobank [https://www.ukbiobank.ac.uk] and GLGC [http://lipidgenetics.org/]) have existing approvals from their respective institutional review boards. All participants provided written informed consent. Full information and references for data sources can be found in Table S1. The current study is reported in accordance with the MR STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (Data S1).

MR Assumptions

MR uses SNPs as instrumental variables (IVs) to identify associations between the genetic liability for an exposure trait and an outcome.[43,45,46] MR has 3 main assumptions (Figure S1): (1) the IV itself must be associated with the exposure (the relevance assumption); (2) there must not exist any causes of the IV that also affect the outcomes through mechanisms other than the exposure of interest (the exchangeability assumption); and (3) the IV must not affect the outcome through a mechanism independent of the exposure, nor affect another trait with a downstream effect on the outcome of interest (the exclusion restriction assumption).[45,46]

The current study used drug-target MR, a recent extension of MR, leveraging variants located within the genomic region of a druggable gene (*cis*-instrumentation) to evaluate whether modulation of a specific drug target (ie, PCSK9) will have an impact on the outcomes of interest (ie, neuropsychiatric end points).[43] This interpretation is different than conventional biomarker MR analyses, which investigates the causal impact of the biomarker (eg, circulating LDL-C levels) on the outcomes of interest.[43] Drug-target MR using *cis*-instruments are less prone to bias because of horizontal pleiotropy.[43] Nevertheless, we included additional MR methods used to assess the sensitivity of our results to different patterns of violations of IV assumptions, which we describe in the Statistical Analysis section.

PCSK9 Instruments

To proxy therapeutic inhibition of PCSK9, we included several separate genetic models incorporating data at the biomarker, protein, and gene expression levels. A brief summary of the PCSK9 instruments used in the present study can be found in Table 1; detailed instrument descriptions are presented in Table S2. First, because the primary physiological response of pharmacological PCSK9 inhibition is lowering circulating levels of LDL-C,[44] as has been done in previous MR studies of PCSK9 and other lipid-lowering target genes,[39,47,48] we extracted SNPs located within 100 kb of the *PCSK9* gene locus (chromosome 1:55505221–55 530 525 GRCh37/hg19) associated with LDL-C levels from participants of European ancestry in the 2021

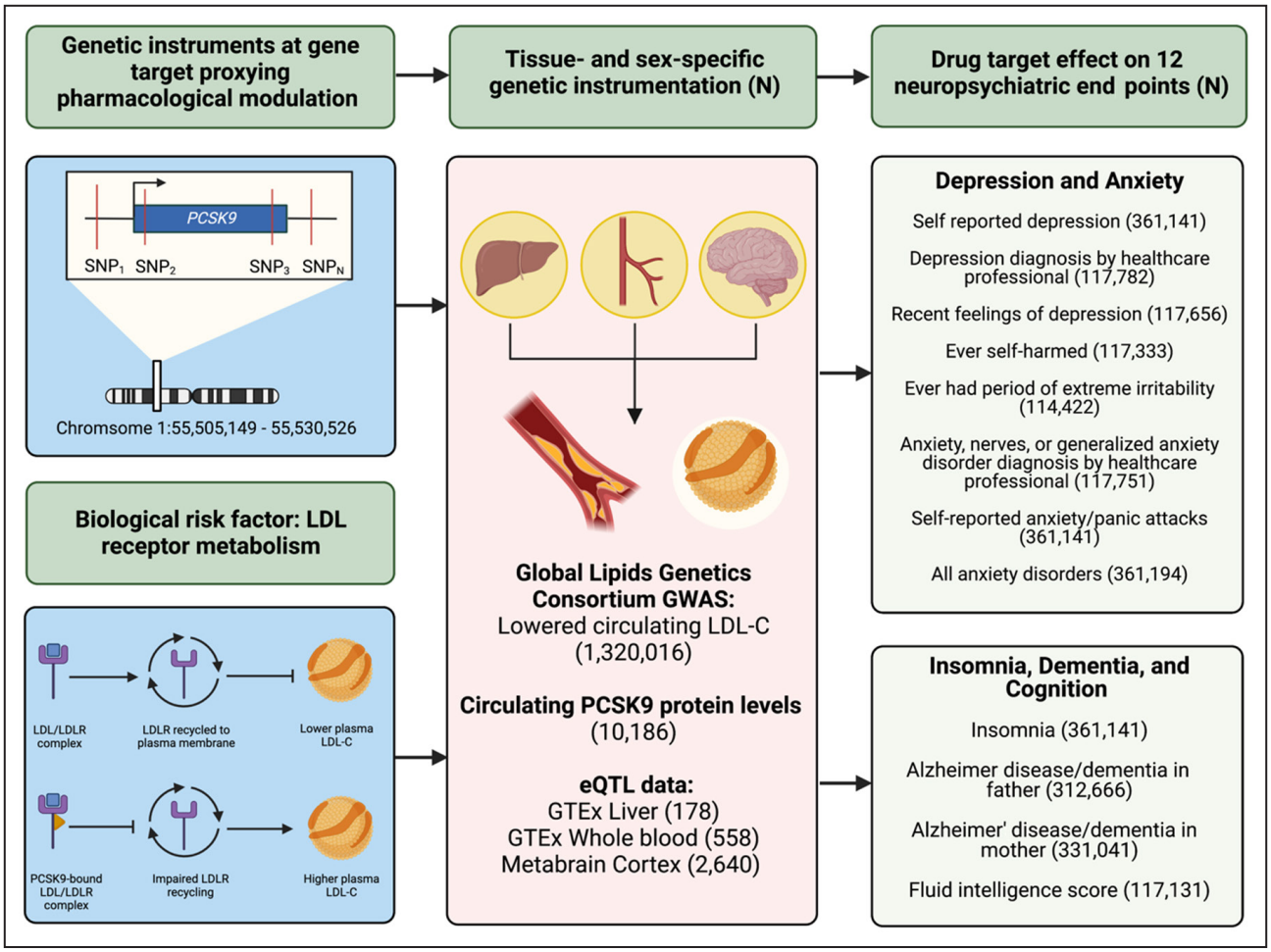

Figure 1. Overview of study methods and procedures.
All summary-level genetic associations were derived from genome-wide association studies (GWAS) of European ancestry. Additional information regarding the GWAS data (consortium, study cohort, and author information of the GWAS for the exposure and neuropsychiatric outcomes) are located in Table S1. We performed *cis*-instrumentation of genetically predisposed PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibition in several complementary data sets. First, single-nucleotide polymorphisms (SNPs) ±100 kilobases of the *PCSK9* gene locus were extracted from the Global Lipids Genetics Consortium (GLGC) 2021 meta-analysis on circulating low-density lipoprotein (LDL) cholesterol (LDL-C) levels surpassing conventional genome-wide significance ($P<5\times10^{-8}$). We also proxied PCSK9 inhibition using circulating levels of the PCSK9 protein and tissue-specific gene expression of *PCSK9* in the liver, whole blood, and brain (cortex). These PCSK9 SNPs were then extracted from selected neuropsychiatric end points spanning mood disorders, insomnia, dementia, and cognition from UK Biobank data that combined men and women, as well as male-only and female-only GWASs. Finally, we performed drug-target Mendelian randomization to evaluate the neuropsychiatric impact of genetically predisposed PCSK9 inhibition across men and women (see Methods section). eQTL indicates expression quantitative trait loci; GTEx Genotype-Tissue Expression; and LDLR, low-density lipoprotein receptor.

GLGC LDL-C European meta-analysis (N≤1 320 016)[49] at conventional genome-wide significance $P<5\times10^{-8}$.[49] We clumped SNPs at linkage disequilibrium $R^2=0.001$, leaving 6 independent SNPs comprising the LDL-C–based PCSK9 instrument. Because only 3 of the 6 independent PCSK9 variants in the LDL-C instrument were found in the PCSK9 protein level data,[50] we also created a PCSK9–LDL-C instrument comprising only variants associated with both LDL-C ($P<5\times10^{-8}$) and PCSK9 protein levels ($P<5\times10^{-6}$),[50] as a biologically conservative instrument and sensitivity analysis to assess the MR exclusion restriction assumption. We included an LDL-C–based PCSK9 instrument with 3 independent SNPs derived from the earlier 2013 GLGC GWAS of circulating LDL-C levels (N≤173 082) as an additional sensitivity analysis.[51] Circulating LDL-C levels are reported in SD units.

Next, because instrumentation of PCSK9 in LDL-C does not measure changes in PCSK9 levels directly, and because current anti-PCSK9 monoclonal antibodies target the PCSK9 protein,[44] we supplemented the PCSK9 instrument derived from LDL-C data with SNPs associated with circulating PCSK9 protein levels using protein quantitative loci (pQTL) data from 10 186

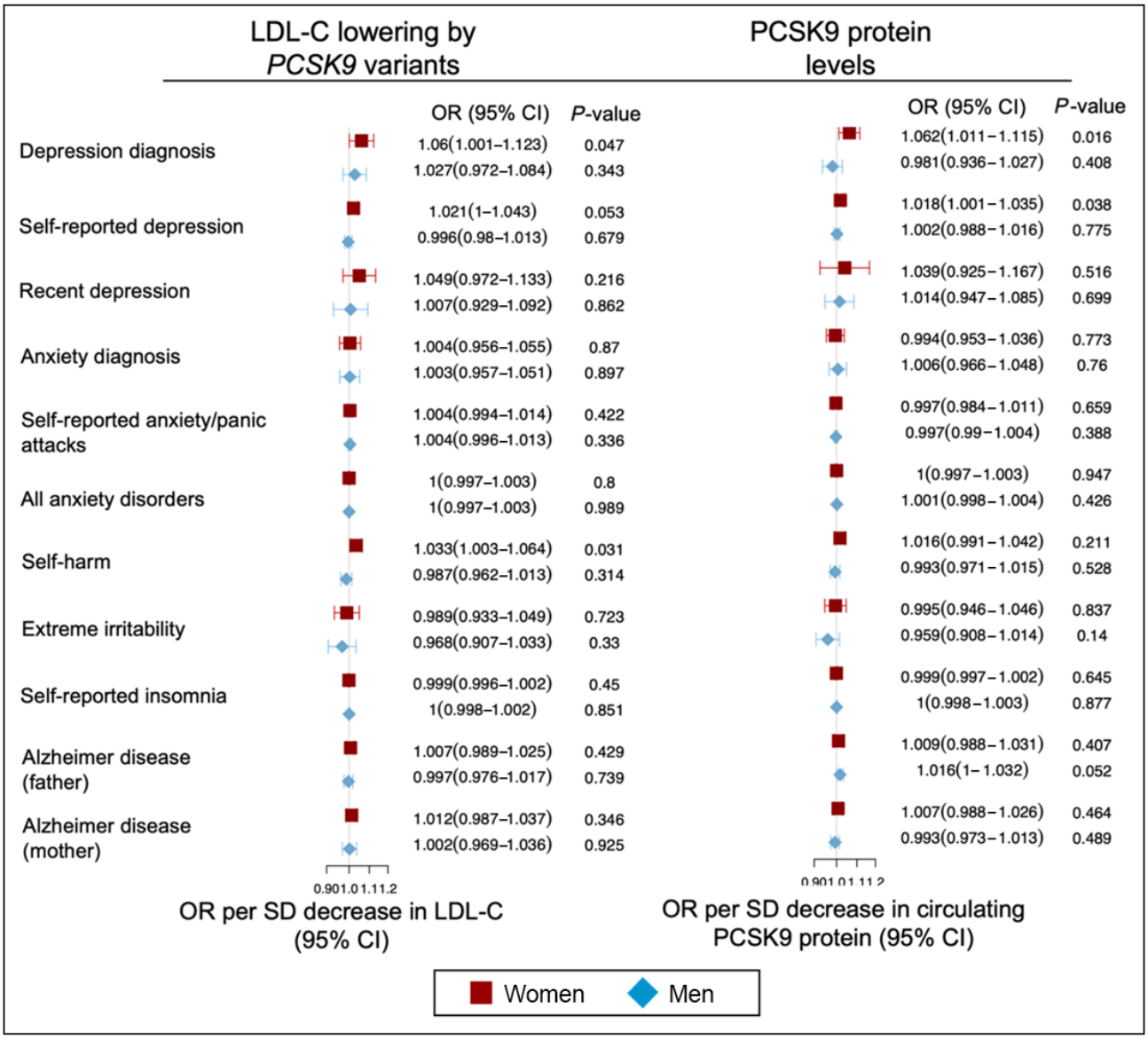

Figure 2. Inverse variance–weighted Mendelian randomization results of genetically proxied PCSK9 (proprotein convertase subtilisin/kexin type 9) in circulating low-density lipoprotein cholesterol (LDL-C) and circulating protein levels on neuropsychiatric outcomes for men and women.

Estimates for the LDL-C–lowering impact of PCSK9 inhibition are reported as odds ratios (ORs) corresponding to a change in the risk for the neuropsychiatric end point per 1-SD reduction of genetically determined circulating LDL-C levels (ie, the primary physiological response of pharmacologic PCSK9 inhibition). For the analyses using circulating PCSK9 protein levels, the ORs correspond to a change in genetically determined normalized circulating PCSK9 protein levels (ie, the primary physiological target of monoclonal PCSK9 inhibitors). Because fluid intelligence is a continuous variable, it was not included in the Forest plot but is discussed in the Results section. Full results, including combined-sex results, are presented in Table S4 and Table S5.

individuals of European ancestry.[50] We extracted and clumped SNPs as above, leaving 2 independent SNPs within the *PCSK9* locus. PCSK9 protein levels were measured in normalized protein units.[50] Given the liver-specific mechanism of action of recently approved small interfering RNA inhibitor, inclisiran,[15] we extracted eQTL from GTEx version 8[52] *PCSK9* data–derived liver tissue (N=178). We *cis*-instrumented the liver *PCSK9* instrument (clumped at linkage disequilibrium R^2=0.001), leaving one variant. Finally, we supplemented these eQTL analyses with additional *PCSK9* instruments from *PCSK9* data derived from whole blood (N=558), and the brain tissue from the MetaBrain analysis (N=2640).[53] The whole blood eQTL *PCSK9* instrument had 2 independent SNPs and the eQTL *PCSK9* instrument from cortex had 1 independent SNP (Table S2). eQTL data are measured in transcripts per million (TPM).[53,54]

Table 1. Summary of PCSK9 Instruments Included in the Current Study

PCSK9 instruments	No. of genetic variants (SNPs)	Average sample size (minimum, maximum)	Average *F* statistic (minimum, maximum)	Total R^2
Circulating LDL-C levels				
PCSK9 SNPs in LDL-C (GLGC 2021[49])	6	1 196 479 (1 094 709, 1 228 324)	484.95 (83.35, 913.76)	0.0024
PCSK9 SNPs in LDL-C (GLGC 2013[51])	3	81 322 (77 417, 86 399)	308.17 (21.66, 762.37)	0.0118
PCSK9 SNPs in LDL-C with variants also associated with PCSK9 protein	2	1 229 493 (1 227 744, 1 231 241)	1206.53 (798.60, 1614.53)	0.00196
Circulating PCSK9 protein levels (pQTL)				
PCSK9 in whole blood	2	9905 (9623, 10 186)	153.42 (92.24, 207.20)	0.03001
Tissue-specific PCSK9 expression (eQTLs)				
PCSK9 in liver	1	178 (NA)	26.83 (NA)	0.1323
PCSK9 in whole blood	3	558 (NA)	44.21 (24.87, 58.54)	0.1374
PCSK9 in cortex	1	2640 (NA)	96.96 (NA)	0.0355

#Genetic variants were the number of PCSK9 (proprotein convertase subtilisin/kexin type 9) single-nucleotide polymorphisms (SNPs) within ±100 kilobases of the *PCSK9* locus included in the instrument before harmonization with the neuropsychiatric outcome data. Average sample size reports the average genome-wide association studies (GWAS) summary statistics sample size for each PCSK9 instrument SNP. Average *F* statistics for each *PCSK9* variant in the instrument. Total R^2 is the variance explained in the GWAS summary statistics by all of the PCSK9 SNPs for each instrument. PCSK9 quantitative trait loci data were obtained from the Pott et al meta-analysis of circulating PCSK9 protein, Genotype-Tissue Expression (GTEx) project portal, and MetaBrain Consortia (links provided in Table S2). Additional PCSK9 instrument information is presented in Table S2. eQTL indicates expression quantitative trait loci; GLGC, Global Lipid Genetics Consortium; LDL-C, low-density lipoprotein cholesterol; and pQTL, protein expression quantitative trait loci.

To test the MR relevance assumption and because MR analyses may be biased by the inclusion of weak instrument SNPs, which may occur when the variants comprising the MR instrument explain only a small proportion of the exposure, resulting in reduced statistical power to reject the null hypothesis,[55] we tested the strength of each PCSK9 instrument by calculating the variance explained by the instrument (ie, the R^2) and the corresponding *F* statistics.[56] By convention, SNP *F* statistics >10 provide evidence that the instruments are unlikely to be subject to weak instrument bias.[56] Every PCSK9 SNP used in this study had estimated *F* statistics exceeding 20 (Table S2). *F* statistics for PCSK9 SNPs within the 2021 LDL-C data ranged from 72.52 to 913.71 (average *F* statistic=484.49). *F* statistics for pQTL and eQTL PCSK9 instruments were similarly strong (minimum, maximum pQTL: 92.24, 207.20; whole blood eQTL: 24.47, 58.54; liver eQTL: 26.83). These *F* statistics indicate minimal bias from weak instruments in the MR analyses.[55]

Circulating Lipid Levels

LDL-C levels have been implicated in neuropsychiatric disorders,[57–59] and because LDL-C is the primary biomarker measured with PCSK9i,[44] we also evaluated the relationships of LDL-C levels and neuropsychiatric outcomes using a polygenic LDL-C instrument. To proxy LDL-C levels, we extracted 400 independent (linkage disequilibrium R^2 <0.001) variants associated with LDL-C at conventional genome-wide significance, irrespective of their genomic position, from the GLGC meta-analysis (N≤1 320 016).[49] A full list of variants used for the polygenic LDL-C instrument is available in Table S3. Instrument SNPs had strong *F* statistics (average *F* statistic, 206.32; range, 29.73–4799.60).

Neuropsychiatric Outcomes From the UK Biobank

We obtained summary-level GWAS data from end points related to depression, anxiety, cognition, and dementia from the UK Biobank for the sex-specific analysis (http://www.nealelab.is/uk-biobank).[60] Additional information for all end points are available in Figure 2 and Table S1. These UK Biobank data sets were derived from participants of European ancestry (ages 40–69 years at the start of data collection). For depression and anxiety, we included self-reported depression (men: 7156 cases/159 832 controls; women: 13 492 cases/180 661 controls); whether the participant reported ever having depression diagnosed by a healthcare professional (men: 8166 cases/43 675 controls; women: 16 921 cases/49 020 controls); whether the participant reported ever having self-harmed (men: 1594/50 262; women: 3505/62 372); whether the participant reported ever having a period of extreme irritability (men: 12 626 cases/37 987 controls; women: 17 121 cases/46 778 controls); whether the participant reported ever having anxiety, nerves, or generalized anxiety disorder diagnosed by a professional healthcare worker (men: 5649 cases/46 176 controls; women: 11 081 cases/54 845 controls); and self-reported anxiety/panic attacks (men: 1813 cases/165 175 controls; women: 3148 cases/191 005 controls).

Given the average age of UK Biobank participants, there are few cases of AD among UK Biobank participants. Therefore, we leveraged the high heritability of

AD, which implies that AD case status for offspring can be, to some extent, inferred by parental AD case status (ie, offspring of parents with AD may have higher genetic risk for AD)[60] and used a phenotype-by-proxy approach with GWAS data on parental AD among participants in the UK Biobank. We used AD status of both fathers (men: 6617 cases/135 280 controls; women: 8405 cases/162 364 controls) and mothers (men: 12 324 cases/136 664 controls; women: 16 183 cases/165 870 controls). Finally, we used a continuous measure of fluid intelligence (men=54 321; women=62 810). For all end points, we analyzed the male, female, and combined GWAS data (Figure 2 and Table S1). We extracted PCSK9 and polygenic LDL-C SNPs for each instrument from each of the outcome GWAS and then harmonized the exposure and outcome data (ie, aligned effect alleles, β coefficients). All PCSK9 instrument SNPs were found in the outcome GWAS data.

Power Calculations

We performed MR power calculations for the primary analyses based on available outcome sample sizes and the variance explained (R^2) of the PCSK9 and polygenic LDL instruments using the mRnd Shiny App.[61] We used an α level of 0.05 and calculated the statistical power to detect odds ratios (ORs) at 3 separate true effect sizes (0.50, 0.80, and 0.90) and considered analyses sufficiently powered where calculated power exceeded 80%.

Sample Independence

The 2021 GLGC meta-analysis incorporated LDL-C from 440 546 UK Biobank participants.[49] For neuropsychiatric outcomes included in this study, the Neale Lab release of the UK Biobank had samples ranging from N=114 422 to N=361 114. Therefore, for analyses with the LDL-C instruments, there is an up to 27% overlap for these combined-sex analyses. However, the maximum overlap for male and female analyses is 12.7% and 14.7%, respectively. While sample overlap in summary-level GWAS data used to estimate genetic associations between exposure and outcome 2-sample MR may potentially bias results,[62,63] any bias would likely be minimal,[62,63] and it has also been shown that 2-sample MR may be safely used in single samples when the data are derived from large biobanks, such as the UK Biobank.[64] We report no sample overlap for the 2013 GLGC LDL-C, PCSK9 pQTL, and GTEx eQTL analyses.

Statistical Analysis

We performed all analysis in R version 4.0.2 using the *TwoSampleMR* R package.[41] For instruments with 2+ SNPs (ie, PCSK9 variants in LDL-C, PCSK9 pQTL, *PCSK9* eQTL in whole blood, and the polygenic LDL-C instrument), we used the inverse variance–weighted (IVW) analyses as the primary MR method. For analyses with instruments comprised of a single SNP (*PCSK9* liver and cortex eQTLs), we used the Wald ratio method as the primary method.[65] For analyses with >2 SNPs (PCSK9 variants associated with LDL-C levels and the polygenic LDL-C analyses), MR Egger, weighted median, and weighted mode analyses are presented as sensitivity analyses to assess the robustness of the MR IVW results and evaluate the exclusion restriction MR assumption. It is assumed that MR IVW gives consistent estimates when all genetic variants are valid IVs.[41,66] Compared with the MR IVW method, MR Egger uses a relaxed assumption rather than the strict MR assumption of no pleiotropy (the Instrument Strength Independent of Direct Effect [InSIDE] assumption).[66,67] MR Egger extends MR IVW by not setting the linear regression intercept to zero, allowing the average horizontal pleiotropic MR estimate across all SNPs to be unbalanced or directional, ie, some variants may be acting on the neuropsychiatric outcomes via ≥1 pathways other than through the PCSK9 or LDL-C exposures.[66,67] The weighted median method uses the median association of all available instrument SNPs.[45] Therefore, only half of the SNPs need to be valid instruments, ie, variants with no horizontal pleiotropy, no associations with confounders, and robust associations with the exposure, to return an unbiased MR estimate.[68] The weighted mode method weights the contribution of each instrument SNP to the clustering by the inverse variance of its association with each neuropsychiatric outcome. Assuming the most common MR instrument is consistent, the overall MR estimate will be unbiased, even if all other SNPs within the instrument are invalid.[45]

While weighted median estimates generally are nearly as precise as MR IVW estimates, both are substantially more precise than MR Egger estimates, with MR Egger estimates particularly imprecise if all IVs have similar exposure effect sizes.[68] Complementary MR methods help evaluate the sensitivity of the results to different patterns of violations of IV assumptions, including horizontal pleiotropy,[45] and consistency of MR estimates across all methods suggests an unbiased MR estimate,[41,42,45] strengthening causal inference.[41] In addition, for the PCSK9 target instrumented in LDL-C and polygenic LDL-C instrument, we also used the MR Egger intercept test,[69] Cochran *Q* heterogeneity test,[70] MR Lasso test,[71] and MR Steiger test[56] assessing causal direction between hypothesized circulating LDL-C and depression, anxiety, cognition, and dementia outcomes.

Because the primary physiological response of pharmacological PCSK9 inhibition is lowered LDL-C

levels, we validated our pQTL and eQTL instruments by investigating their impact on LDL-C. To simulate the pharmacological impact of PCSK9 inhibitors, the reported drug-target MR estimates were transformed to correspond to a decrease of LDL-C in units of SD, circulating PCSK9 protein levels, and lower *PCSK9* gene expression. For instruments derived from LDL-C levels, this corresponds to a change in the likelihood of reporting a positive neuropsychiatric end point per a 1-SD decrease in circulating LDL-C levels. For the PCSK9 protein and gene expression analyses, this instead corresponds to a change in the likelihood per a 1-SD change in normalized PCSK9 protein levels and TPM, respectively. We report 95% CIs estimates for the MR analyses. The strength of evidence was indexed against the null hypotheses (no association) by the exact *P* value before and after correction for multiple testing. To account for multiple testing bias, a Bonferroni correction was used, where the adjusted *P* value threshold was 4.17×10^{-3} (0.05/12 end points tested). For any nominally significant findings ($P<0.05$) in the sex-specific end points, a post hoc hypothesis test was performed to evaluate whether the MR estimates between the male and female end point were significantly different (*P* value <0.05) from each other.

RESULTS

Impact of LDL-C Lowering by PCSK9 on Neuropsychiatric Risk

Drug-target MR analyses proxying genetic PCSK9 inhibition in LDL-C levels failed to find any IVW MR estimates among either men or women surpassing Bonferroni correction for multiple comparisons (Figure 2). Full results are presented in Table S4 and power calculations in Table S5. MR estimates (β) for fluid intelligence were 0.0246 ($P=0.877$) and – 0.0048 ($P=0.644$) for men and women, respectively. For women, genetically predisposed PCSK9 inhibition was associated with a nominally significant increase in the risk of self-harm (OR, 1.003; $P=0.031$). Genetically predisposed PCSK9 inhibition was also associated with depression diagnosis (OR, 1.06; $P=0.047$). MR estimates for men for these end points were null (self-harm OR, 0.99; $P=0.313$; depression OR, 1.023; $P=0.343$); however, the post hoc hypothesis test revealed no statistically significant difference between the male and female estimates ($P=0.091$) (Table S4).

Results using the PCSK9 instrument derived from 2013 GLGC LDL-C data were generally aligned (Table S6); however, associations with self-harm in women (OR, 1.025; $P=0.155$) were not statistically significant. Overall, these results were consistent across MR methods. MR Egger intercept analysis suggested no evidence of horizontal pleiotropy and the Cochran *Q* test did not indicate heterogeneity, which improves the causal inference of the MR estimates.[45]

Further, analysis of circulating LDL-C levels proxied by instruments comprised of SNPs throughout the genome did not yield evidence for any association with depression risk in women or men (Table S7). We did observe one end point surpassing correction for multiple comparisons: in the combined-sex analysis, increased LDL-C levels were associated with decreased risk for paternal AD risk (OR, 0.995; $P=0.0013$) but not maternal AD ($P=0.029$).

Assessing the Neuropsychiatric Impact of PCSK9 QTL Instruments

MR testing validity of the QTL PCSK9 instruments by evaluating their causal impact on circulating LDL-C levels showed that increased genetically proxied PCSK9 inhibition was associated with lowered LDL-C (Table S8). Our pQTL and tissue-specific eQTL results aligned with the LDL-C–based PCSK9 analyses failing to find evidence of a neuropsychiatric impact of genetically proxied PCSK9 inhibition and, as before, we observed nominally significant increased risk for self-reported depression in women (Table S9), ie, an OR of 1.018 ($P=0.038$) for reduced circulating PCSK9 protein (Figure 2), an OR of 1.0094 ($P=0.0064$) for liver *PCSK9* expression, and an OR of 1.024 ($P=0.036$) for whole blood *PCSK9* expression (Figure 3). Genetically proxied circulating PCSK9 protein inhibition was associated with an increased risk for depression diagnosis in women (OR, 1.062; $P=0.016$). Post hoc hypothesis testing found a statistical difference between men and women in self-reported depression for liver PCSK9 expression ($P=0.035$) but not for whole blood genetic *PCSK9* expression ($P=0.18$) or circulating genetic PCSK9 protein ($P=0.12$).

Power Calculations

Power calculations showed that the eQTL and pQTL analyses were generally sufficiently powered (power exceeding 80%) to detect the presence of ORs of ≥1.2 at a type 1 error rate (α level) of 0.05 for the combined-sex and sex-specific analyses for all neuropsychiatric outcomes except for self-reported anxiety/panic attacks (Table S9). Broadly, PCSK9 instruments using eQTL and pQTL data were better powered than the PCSK9 instruments derived from circulation LDL data because of the increased variance explained by the instruments (eg, PCSK9 liver eQTL variants explained 13.74% of the variance in PCSK9 liver expression compared with 0.242% of the variance of LDL-C levels [Table S9]). As expected, the estimated power to detect ORs of ≤1.1 was reduced; however, the eQTL- and pQTL-based analyses remained well powered for

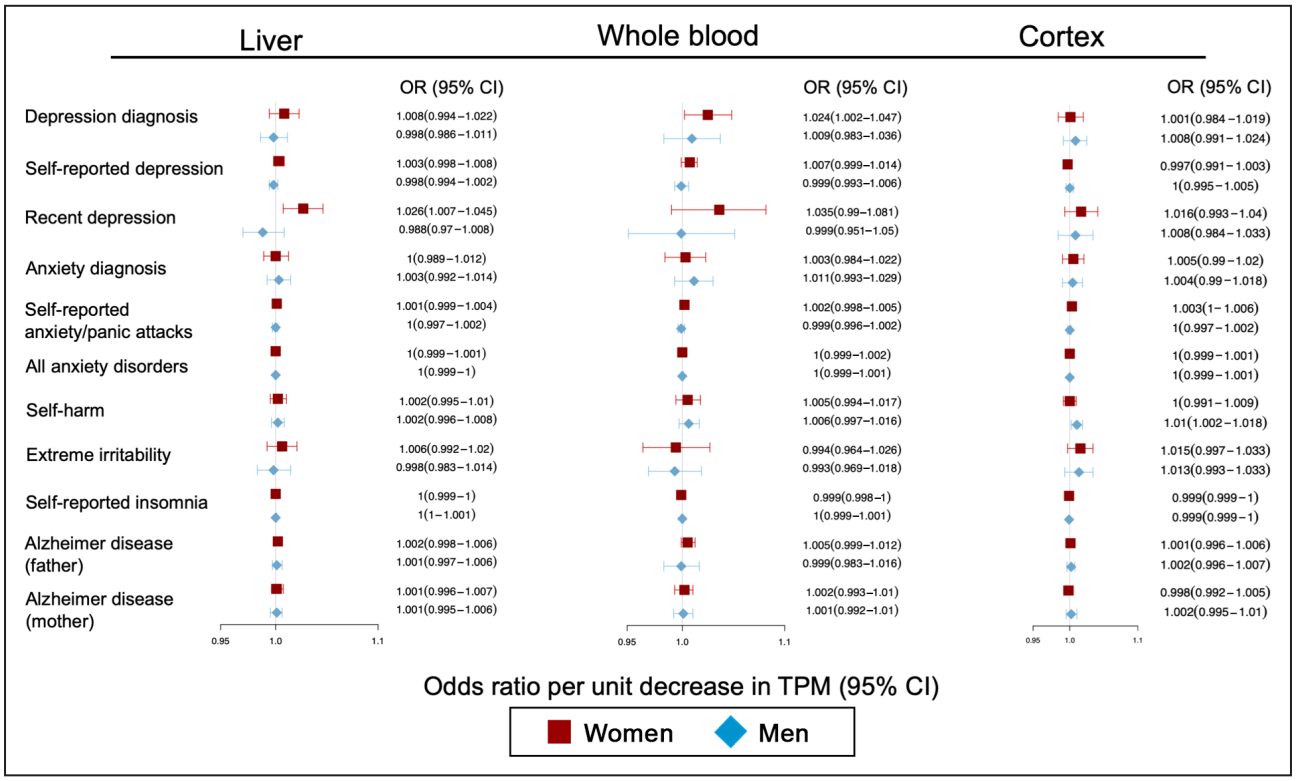

Figure 3. Inverse variance–weighted and Wald ratio Mendelian randomization results of genetically proxied lowering of tissue-specific PCSK9 (proprotein convertase subtilisin/kexin type 9) gene expression on neuropsychiatric outcomes for men and women.

Estimates are reported odds ratios (ORs) and 95% CIs corresponding to a change in the risk for the neuropsychiatric end point for a change in genetically determined liver, whole blood, and brain *PCSK9* gene expression (measured in transcripts per million [TPM]). Full results, including the combined-sex results, are presented in Table S9.

several end points, including irritability, depression, cognition, and AD (Table S5).

DISCUSSION

The present study found that the neuropsychiatric impact of genetically proxied inhibition of PCSK9 was both generally neutral and similar between men and women, adding to the growing body of literature suggesting a safe neuropsychiatric profile for PCSK9 inhibitors and other lipid-lowering therapies.[13,21–23,38,72,73] Our results provide an important sex-specific comparison of potential neuropsychiatric adverse side effects related to genetically proxied PCSK9i, which has not been heretofore evaluated despite the call for more studies stratifying analyses by sex[30] and reported differences in prevalence of these disorders between men and women.[31–36] Overall, these results may help mitigate ongoing fears of adverse neuropsychiatric side effects related to PCSK9i that have contributed, in part, to their underutilization.[74]

We did observe some associations between genetically proxied PCSK9 inhibition and depression, which adds to the growing body of MR literature suggesting a causal relationship between depression and cardiovascular disease.[75–77] Notably, our corresponding null estimates on depression risk from polygenic LDL-C instruments using variants across the genome suggest that the relationship is not mediated by the primary physiological response to PCSK9 inhibition. Further, while some instruments suggested a nominal statistical increase in the risk of depression, other instruments suggested potential beneficial effects of PCSK9 inhibition (eg, the biologically conservative PCSK9 instrument). Further, causal inference requires triangulating study designs,[78] and an impact by PCSK9i on depression has not been observed in RCTs or observational data,[13,21–23,38,72] suggesting more work is needed to further elucidate the direction of these relationships.

The present study made use of the ability of drug target MR to estimate the causal relationships between a modifiable exposure (PCSK9 levels) and neuropsychiatric outcomes of interest by examining the association between exposure-associated genetic variants, as IVs, and the outcome of interest. Analyses of participant data from short-term RCTs have used data from patients on concurrent statin therapy,[13,22] potentially complicating evaluation of the neuropsychiatric impact

specifically related to PCSK9i; however, the MR framework can estimate these relationships while minimizing the impact of confounding variables,[41,42] allowing use of population-based observational data to strengthen causal inference regarding the long-term neuropsychiatric effects of PCSK9i. We also leveraged new data sources measuring the genetic component of *PCSK9* gene expression and protein levels in addition to liver-specific *PCSK9* gene expression,[50] which, in addition to providing multiple PCSK9 instruments to evaluate potential neuropsychiatric effects, may also yield important information regarding specific PCSK9 drug classes (ie, approved anti-PCSK9 monoclonal antibodies that target circulating PCSK9 protein levels,[44] while inclisiran targets liver-specific PCSK9 expression[15]). Further, the use of PCSK9 instruments from gene expression, protein levels, and biomarkers reduces the risk of reverse causation and can provide important complementary evidence in drug-target MR studies.[43]

We note several limitations and important factors for interpretation. These results represent the analysis of only summary statistics taken from large, public GWAS data sets. As such, no clinical data or data from RCTs were included in this analysis. While MR is a powerful method for assessing genetic relationships, it is not a substitute for RCTs, which remain the gold standard for assessing causal biological relationships. Additionally, causal inference in MR depends on several assumptions that cannot be verified (eg, the exchangeability and exclusion restriction assumptions),[45] and while *cis*-instrument MR is less prone to horizontal pleiotropy,[43,48] and sensitivity analyses yielded consistent MR estimates across all methods employed in the study, the possibility of bias caused by confounding or pleiotropy cannot be completely disregarded. Further, our *cis*-instrument made use only of those SNPs in the *PCSK9* gene associated with LDL-C, and therefore did not assess for any effects of PCSK9 inhibition through alternative pathways. Additional research in real-world settings, including long-term studies of postmarketing data, are needed to address the remaining uncertainties regarding PCSK9 inhibition. Relatedly, drug-target MR cannot itself proxy any potential off-target effects of specific drug classes, such as adverse effects of the RNAi delivery system used for treatment with inclisiran. Moreover, MR estimates assess preexisting, permanent genetic variants with permanent biological effects beginning in early development. In contrast, PCSK9 inhibitors are generally prescribed to adults; the temporal difference in the effective developmental stage of the 2 populations represents a limitation of drug-target MR methods for completely proxying pharmacological outcome profiles.[41,45] Therefore, we urge caution regarding the interpretation of these results for clinical decision-making, pending replication and further investigation.[79] In addition, the current study outcomes were based on self-reported data, including self-reported diagnoses. Self-reported outcomes in the behavioral and healthcare literature are subject to response bias, either due to underreporting or overreporting,[80] which might also impact the analyses.

There are additional limitations related to statistical power and sample overlap. While power calculations suggested the analyses were generally sufficiently powered to detect the evidence of the potential neuropsychiatric impact of genetically predisposed PCSK9 inhibition, some analyses using the PCSK9 variants instrumented in the LDL-C data, despite strong *F* statistics, may be underpowered. Nevertheless, power calculations of analyses of these same end points using pQTL and eQTL instruments were well-powered, which improves the causal inference of the null MR estimates. In addition, while there was no sample overlap between the 2013 LDL-C, pQTL, and eQTL analyses, the 2021 GLGC data included UK Biobank participants,[49] which may bias these estimates.[63] Recent work has shown that 2-sample MR may safely be used in single samples (ie, 100% sample overlap) provided the data are derived from large biobanks, such as the UK Biobank,[64] so any sample overlap bias is likely minimal. Finally, because our analyses were limited to participants of European ancestry, including outcomes drawn exclusively from the UK Biobank, which has been shown to be more educated and generally healthier than the general UK population,[81] caution is warranted before generalizing our findings to other populations; replication is needed in populations of other ancestries when such data become available.

CONCLUSIONS

Our drug-target MR analysis of genetically proxied PCSK9 inhibition suggests a similar and neutral neuropsychiatric profile for PCSK9i therapy in men and women. While it is possible that the lack of statistically significant findings for certain end points may be attributable to insufficient power, our findings generally aligned across the neuropsychiatric end points using PCSK9 instruments derived from gene expression data, circulating PCSK9 protein levels, and PCSK9 locus-lowering of LDL-C. Future studies with larger, more diverse GWAS data, and additional long-term and postmarketing research will continue to further our understanding of the possible neuropsychiatric impact of PCSK9 inhibition.

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Disclosures

None.

Supplemental Material

Data S1
Figure S1
Table S1–S9

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SUPPLEMENTAL MATERIAL

Data S1. STROBE-MR Reporting Guidelines

1. TITLE and ABSTRACT

Indicate Mendelian randomization as the study's design in the title and/or the abstract.

Mendelian randomization is present in both the title and abstract of the manuscript.

INTRODUCTION

2. Background

Explain the scientific background and rationale for the reported study. Is causality between exposure and outcome plausible? Justify why MR is a helpful method to address the study question.

Addressed in the Introduction and Methods section of the main manuscript.

3. Objectives

State specific objectives clearly, including pre-specified causal hypotheses (if any).

Addressed in the Introduction section of the main manuscript.

METHODS

4. Study design and data sources

Present key elements of study design early in the paper. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:

a) Describe the study design and the underlying population from which it was drawn.

Describe also the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, if available.

b) Give the eligibility criteria, and the sources and methods of selection of participants.

c) Explain how the analyzed sample size was arrived at.

d) Describe measurement, quality and selection of genetic variants.

e) For each exposure, outcome and other relevant variables, describe methods of assessment and, in the case of diseases, the diagnostic criteria used.

f) Provide details of ethics committee approval and participant informed consent, if relevant.

Addressed in the Methods section of the main manuscript.

5. Assumptions

Explicitly state assumptions for the main analysis (e.g. relevance, exclusion, independence, homogeneity) as well assumptions for any additional or sensitivity analysis.

Addressed in the Methods section of the main manuscript.

6. Statistical methods: main analysis

Describe statistical methods and statistics used.

a) Describe how quantitative variables were handled in the analyses (i.e., scale, units, model).

b) Describe the process for identifying genetic variants and weights to be included in the analyses (i.e, independence and model). Consider a flow diagram.

c) Describe the MR estimator, e.g. two-stage least squares, Wald ratio, and related statistics.

Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples.

d) Explain how missing data were addressed.

e) If applicable, say how multiple testing was dealt with.

Addressed in the Methods section of the main manuscript.

7. Assessment of assumptions

Describe any methods used to assess the assumptions or justify their validity.

Addressed in the Methods section of the main manuscript.

8. Sensitivity analyses

Describe any sensitivity analyses or additional analyses performed.

Addressed in the Methods section of the main manuscript.

9. Software and pre-registration

a) Name statistical software and package(s), including version and settings used.

Addressed in the Methods section of the main manuscript.

b) State whether the study protocol and details were pre-registered (as well as when and where).

Addressed in the Methods section of the main manuscript.

RESULTS

10. Descriptive data

a) Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow-diagram.

b) Report summary statistics for phenotypic exposure(s), outcome(s) and other relevant variables (e.g. means, standard deviations, proportions).

c) If the data sources include meta-analyses of previous studies, provide the number of studies, their reported ancestry, if available, and assessments of heterogeneity across these studies. Consider using a supplementary table for each data source.

d) For two-sample Mendelian randomization:

i. Provide information on the similarity of the genetic variant-exposure associations between the exposure and outcome samples.

ii. Provide information on extent of sample overlap between the exposure and outcome data sources.

Addressed in the Methods and Results sections of the main manuscript and Supplemental Tables.

11. Main results

a) Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale (e.g. comparing 25th and 75th percentile of allele count or genetic risk score, if individual-level data available).

b) Report causal effect estimate between exposure and outcome, and the measures of uncertainty from the MR analysis. Use an intuitive scale, such as odds ratio, or relative risk, per standard deviation difference.

c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time-period.

d) Consider any plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure).

Addressed in the Results section of the main manuscript and Supplemental Tables.

12. Assessment of assumptions

a) Assess the validity of the assumptions.

b) Report any additional statistics (e.g., assessments of heterogeneity, such as I2, Q statistic).

Addressed in the Results and Discussion section of the main manuscript and Supplemental Tables.

13. Sensitivity and additional analyses

a) *Use sensitivity analyses to assess the robustness of the main results to violations of the assumptions.*

b) Report results from other sensitivity analyses (e.g., replication study with different dataset, analyses of subgroups, validation of instrument(s), simulations, etc.).

c) Report any assessment of direction of causality (e.g., bidirectional MR).

d) When relevant, report and compare with estimates from non-MR analyses.

e) Consider any additional plots to visualize results (e.g., leave-one-out analyses).

Addressed in the Results section of the main manuscript and Supplemental Tables.

DISCUSSION

14. Key results

Summarize key results with reference to study objectives.

Addressed in the Discussion section of the main manuscript.

15. Limitations

Discuss limitations of the study, taking into account the validity of the MR assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias, and any efforts to address them.

Addressed in the Discussion section of the main manuscript.

16. Interpretation

a) Give a cautious overall interpretation of results considering objectives and limitations.

Compare with results from other relevant studies.

b) Discuss underlying biological mechanisms that could be modelled by using the genetic variants to assess the relationship between the exposure and the outcome.

c) Discuss whether the results have clinical or policy relevance, and whether interventions could have the same size effect.

Addressed in the Discussion section of the main manuscript.

17. Generalizability

Discuss the generalizability of the study results (a) to other populations (i.e. external validity),

(b) across other exposure periods/timings, and (c) across other levels of exposure.

Addressed in the Discussion section of the main manuscript.

OTHER INFORMATION

18. Funding

Give the source of funding and the role of the funders for the present study and, if applicable, for the original study or studies on which the present article is based.

Addressed in the Funding section of the main manuscript.

19. Data and data sharing

Present data used to perform all analyses or report where and how the data can be accessed. State whether statistical code is publicly accessible and if so, where.

Addressed in the Methods section of the main manuscript.

20. Conflicts of Interest

All authors should declare all potential conflicts of interest.

Addressed in the Conflicts of Interest section of the main manuscript.

Table S1. Phenotype descriptives and sources

Table S2. PCSK9 instruments

Table S3. Polygenic LDL instrument

Table S4. SVMR results for PCSK9 in LDL-C on sex-stratified neuropsychiatric outcomes aligned with PCSK9 inhibition with the primary 2021 GLGC LDL-C instrument

Table S5. Statistical power to detect odds ratio of OR 2.00, 1.20, and 1.10 per standard deviation decrease in exposure levels at a 5% false positive rate

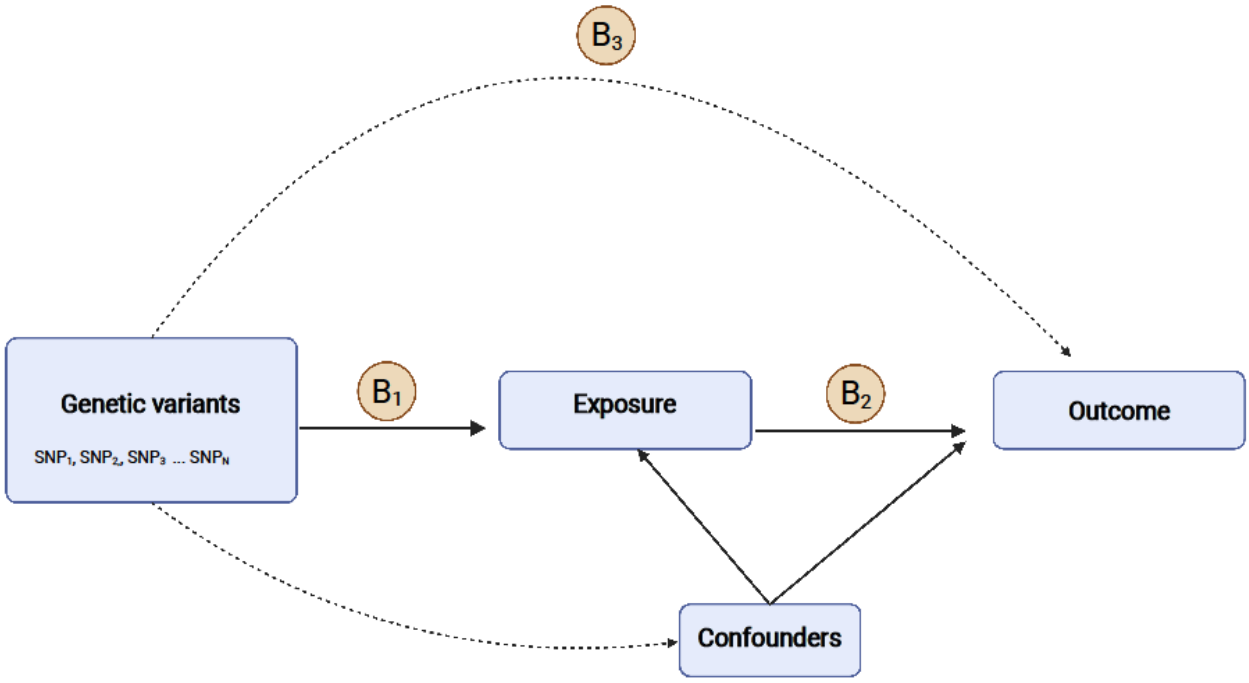
Table S6. Results of sex-specific drug-target MR on all outcomes from secondary PCSK9 LDL-C instrument derived from 2013 GLGC data

Table S7. SVMR results for LDL-C on sex-stratified neuropsychiatric outcomes

Table S8. SVMR results for PCSK9 cis-located instruments in EQTL/liver, EQTL/whole blood, and PQTL/whole blood on LDL-C

Table S9. SVMR results for PCSK9 in liver EQTL, whole blood PQTL, whole blood EQTL, and cortex and meta-brain EQTLs on sex-stratified neuropsychiatric outcomes aligned with PCSK9 inhibition

Figure S1. Mendelian Randomization Model and Assumptions



B_2 is the genetic association of interest, estimated by $B_2=B_1/B_3$. B_1 and B_3 are the associations of the genetic variants with the exposure and the outcome. MR assumes that the genetic variants comprising the instrument for the exposure only impact the outcome of interest via the exposure and not directly, or via confounders (dotted lines).[53]